REMARKS/ARGUMENTS

Claims 58-63, 69 and 70 are pending in this application.

I. Claim Rejections Under 35 U.S.C. §§101 and 112, First Paragraph (Enablement)

Claims 58-63 and 69-70 remain rejected under 35 U.S.C. §101 allegedly "because the claimed invention is not supported by either a specific and substantial asserted utility or a well established utility." (Page 2 of the instant Office Action).

Claims 58-63 and 69-70 further remain rejected under 35 U.S.C. §112, first paragraph, allegedly "since the claimed invention is not supported by either a credible, specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention." (Page 3 of the instant Office Action).

Applicants submit, as discussed below, that not only has the PTO not established a *prima facie* case for lack of utility, but that the polypeptides of Claims 58-63 and 69-70 possess a specific and substantial asserted utility, and that based upon this utility, one of skill in the art would know how to use the claimed polypeptides without any further experimentation.

The gene amplification data disclosed in Example 114 establishes a credible, substantial and specific patentable utility for the PRO213-1 polypeptides.

First of all, Applicants respectfully maintain the position that the specification discloses at least one credible, substantial and specific asserted utility for the claimed PRO213-1 polypeptides for the reasons previously set forth in Applicants' Responses filed on October 4, 2004, May 23, 2005, and November 18, 2005, in the Preliminary Amendment filed July 7, 2006, and in the Supplemental Preliminary Amendment filed September 6, 2006.

Furthermore, as first discussed in Applicants' Response of October 4, 2004, Applicants rely on the gene amplification data for patentable utility of the PRO213-1 polypeptide, and the gene amplification data for the gene encoding the PRO213-1 polypeptide is clearly disclosed in the instant specification under Example 114. As previously discussed, a ΔCt value of at least 1.0 was observed for PRO213-1 in at least 35 of the lung and colon primary tumors and tumor cell lines listed in Table 9. Table 9 teaches that the nucleic acids encoding PRO213-1 showed 1.03 to 5.55 ΔCt units which corresponds to $2^{1.03}$ to $2^{5.55}$ - fold amplification or 2.04 to 46.9 - fold amplification in 16 different human primary lung tumors, LT1, LT1a, LT3, LT4, LT6, LT7, LT9,

LT11, LT12, LT13, LT15, LT16, LT17, LT19, LT21 and LT22. PRO213-1 also showed 1.18 to 3.79 ΔCt units which corresponds to $2^{1.18}$ to $2^{3.79}$ - fold amplification or 2.27 to 13.8 - fold amplification in 11 different human primary colon tumors, CT2, CT4, CT5, CT6, CT8, CT10, CT12, CT14, CT15, CT16 and CT17. In addition, PRO213-1 showed 1.31 to 2.95 ΔCt units which corresponds to $2^{1.31}$ to $2^{2.95}$ - fold amplification or 2.48 to 7.73 - fold amplification in three different lung cancer cell lines (Calu-1, H441 and H810), and 1.22 to 2.08 ΔCt units which corresponds to $2^{1.22}$ to $2^{2.08}$ - fold amplification or 2.33 to 4.23 - fold amplification in five different colon cancer cell lines (HT29, SW403, LS174T, HCT15 and HCC2998).

Accordingly, the present specification clearly discloses overwhelming evidence that the gene encoding the PRO213-1 polypeptide is significantly amplified in a significant number of lung and colon tumors. Thus one of ordinary skill in the art would find it credible that PRO213-1 has utility as a diagnostic marker of lung and colon tumors.

A prima facie case of lack of utility has not been established

The Examiner asserts that the disclosed gene amplification data does not provide utility for the claimed PRO213-1 polypeptides because "[t]he issue is whether or not amplified DNA correlates with increased mRNA, and whether or not increased mRNA correlates with increased protein levels," and allegedly, the art of record establishes "there is no strong connection between gene amplification and increased mRNA or protein levels." (Page 3 of the instant Office Action). In support of this assertion the Examiner has previously cited articles by Pennica *et al.*, Gygi *et al.*, Lian *et al.*, Fessler *et al.*, Chen *et al.* and Anderson *et al.*

The Examiner first refers to the previously cited references by Pennica *et al.* and Gygi *et al.* Applicants respectfully submit that, for the reasons previously set forth in Applicants' Responses filed on October 4, 2004, and May 23, 2005, the teachings of Pennica *et al.* are specific to *WISP* genes, and say nothing about the correlation of gene amplification and protein expression in general.

Gygi *et al.*

Applicants reiterate that, as acknowledged by the Examiner, the data shown in Gygi *et al.* clearly indicates that high levels of mRNA **generally** correlate with high levels of proteins. Gygi *et al.* may teach that protein levels cannot be "predicted" from mRNA levels in the sense that the exact numerical amounts of protein present in a tissue cannot be determined based upon mRNA

levels. Applicants respectfully submit that the PTO's emphasis on the need to "accurately predict" protein levels based on mRNA levels misses the point. The asserted utility for the claimed polypeptides is in the diagnosis of cancer. What is relevant to use as a cancer diagnostic is relative levels of gene or protein expression, not absolute values, that is, that the gene or protein is differentially expressed in tumors as compared to normal tissues. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predict protein overexpression. A showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required.

Applicants further respectfully submit that Futcher *et al.* (Mol. Cell. Biol. 19:7357-7368 (1999); submitted with Applicants' IDS filed July 7, 2006) also analyzed the yeast proteome using 2D gel electrophoresis, gathering quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that "**several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance**" (page 7360, col. 2; emphasis added).

The authors note that Gygi *et al.* completed a similar study that generated broadly similar data, but reached different conclusions. Futcher *et al.* point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." Futcher *et al.* note that Gygi *et al.* used the Pearson product-moment correlation coefficient (r_p) and point out that "a calculation of r_p is inappropriate" because the mRNA and protein abundances are not normally distributed (page 7367, col. 1). In contrast, Futcher *et al.* used two different statistical approaches to determining the correlation between mRNA and protein abundances. First, they used the Spearman rank correlation coefficient (r_s), an nonparametric statistic that does not require the data to be normally distributed. Using the r_s, the authors found that mRNA abundance was well correlated with protein abundance ($r_s = 0.74$). Applying this statistical approach to the data of Gygi *et al.* **also** resulted in a good correlation ($r_s = 0.59$), although the correlation was not quite as strong as for the Futcher *et al.* data. In a second approach, Futcher *et al.* transformed the mRNA and protein data to forms where they were normally distributed, in order to allow calculation of an r_p. Two types of transformation (Box-Cox and logarithmic) were used, and **both** resulted in good correlations between mRNA and protein abundance for Futcher *et al.*'s data.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots.

In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "**the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation *in vivo***" (page 7367, col. 2; emphasis added). Thus while these lowest abundant proteins do show a poor correlation, this is almost certainly due to the less accurate methods used to measure the abundance of these proteins, and **not** to any actual lack of correlation.

Lian *et al.*

Applicants have previously pointed out that Lian *et al.* admit that there are a number of problems with the data presented in this reference. Applicants also emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. As discussed in their Preliminary Amendment filed July 7, 2006, Lian *et al.* did not take genes which showed significant mRNA changes and check the corresponding protein levels. Instead, the authors looked at a small and unrepresentative number of proteins, and checked the corresponding mRNA levels. Based on the authors' criteria, mRNA levels were significantly changed if they were at least 5-fold different when measured using a microchip array, or 2-fold different when using the more sensitive 3'-end differential display (DD). Of the 28 proteins listed in Table 6, only one has an mRNA level measured by microarray which is differentially expressed according to the authors (spot 7: melanoma X-actin, for which mRNA

changed from 2539 to 341.3, and protein changed from 1 to 3). None of the other mRNAs listed in Table 6 show a significant change in expression level when using the criteria established by the authors for the less sensitive microarray technique.

Only two genes meet the authors' criteria for differentially expressed mRNA level, and of those, one apparently shows a corresponding change in protein level and one does not. The Examiner asserts that "even if only two genes met the authors' criteria for differentially expressed mRNA level and one shows a corresponding change in protein level and one does not, one of skill in the art would determine that it is not predictable that a change in mRNA would lead to a corresponding change in protein." (Page 4 of the instant Office Action). Applicants respectfully submit that this is an inappropriate conclusion. One of ordinary skill in the art, seeing that Lian *et al.* provided only two contradictory examples of genes meeting their criteria, would properly conclude that the reference provided insufficient data to draw any conclusions.

Fessler *et al.*

The Examiner asserts that "Fessler is evidence that there is a poor correlation between changes in levels of protein and corresponding changes in levels of mRNA." (Page 5 of the instant Office Action). As discussed in their Preliminary Amendment filed July 7, 2006, Fessler is not contrary to Applicants' asserted utility, and actually supports Applicants' assertion that a change in the level of mRNA for a particular protein generally leads to a corresponding change in the level of the encoded protein. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Applicants' asserted utility.

Chen *et al.* and Anderson *et al.*

The Examiner next discusses the previously cited reference by Chen *et al.* Applicants reiterate that, as discussed in their Response filed November 18, 2005, no attempt was made to compare expression levels in normal versus tumor samples, and in fact the authors concede that they had too few normal samples for meaningful analysis (page 310, col. 2). As a result, the analysis in the Chen paper shows only that a number of randomly selected proteins have varying degrees of correlation between mRNA and protein expression levels within a set of different lung adenocarcinoma samples. The Chen paper does not address the issue of whether increased mRNA levels in the tumor samples taken together as one group, as compared to the normal

samples as a group, correlated with increased protein levels in tumorous versus normal tissue.

Applicants have asserted that an increase in mRNA expression in tumor tissue as compared to normal tissue will, in general, correlate with increased protein expression in the same tumor tissue as compared to normal tissue. Chen *et al.* did not examine the correlation between increases in mRNA and protein expression in tumor tissue as compared to normal tissue and says nothing about it. Accordingly, the results presented in the Chen paper are not applicable to the application at issue.

The Examiner asserts that both Chen *et al.* and Anderson *et al.* support the assertion that "transcript levels do not necessarily correlate with protein levels." (Page 6 of the instant Office Action). Applicants respectfully submit that their assertion is not that transcript levels necessarily correlate in all cases with protein levels, but that, in general, a change in the level of mRNA for a particular gene in tumor as compared to normal tissue leads to a corresponding change in the level of protein encoded by the gene. As discussed above and in Applicants' previous Responses, neither Chen nor Anderson addresses this issue, and thus provide no evidence to contradict Applicants' assertion.

Nagaraja *et al.*, Waghray *et al.*, and Sagynaliev *et al.*

In further support of the assertion that "*changes* in mRNA expression frequently do not result in *changes* in protein expression", the Examiner cites three new references, by Nagaraja *et al.*, Waghray *et al.*, and Sagynaliev *et al.* (Pages 6-7 of the instant Office Action; emphasis in original).

The Examiner cites Nagaraja *et al.* as allegedly teaching that in comparisons of expression profiles for normal breast compared to breast cancer, "the proteomic profiles indicated altered abundance of fewer proteins as compared to transcript profiles." (Page 6 of the instant Office Action).

Applicants respectfully submit that the fact that many more transcripts than proteins were found to be differentially expressed does not mean that most mRNA changes did not result in correlating protein changes, but merely reflects the fact that expression levels were only measured at all for many fewer proteins than transcripts. In particular, the total number of proteins whose expression levels could be visualized on silver-stained gels was only about 300 (page 2332, col. 1), as compared to the approximately 14,500 genes on the microarray chips for which mRNA levels were measured (page 2336, col. 1). Since the expression levels of so many

fewer proteins than transcripts were measured, it is hardly surprising that a smaller absolute number of proteins than mRNAs were found to be overexpressed, because the protein products of most of the overexpressed mRNAs would not have been among the small number of proteins identified on the gels.

The Examiner next cites Waghray *et al.*, to the effect that "for most of the proteins identified, there was no appreciable concordant change at the RNA level." (Page 7 of the instant Office Action).

Applicants emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. Waghray *et al.* did not take genes which showed significant mRNA changes and check the corresponding protein levels. Instead, the authors looked at a small and unrepresentative number of proteins, and checked the corresponding mRNA levels. Waghray *et al.* acknowledge that only "[a] relatively small set of genes could be analyzed at the protein level, largely due to the limited sensitivity of 2-D PAGE" (page 1337, col. 1). In particular, while the authors examined the expression levels of 16,570 genes (page 1329, col. 2), they were able to measure the expression levels of only 1031 proteins (page 1333, col. 2). Waghray *et al.* does not teach that changes in mRNA expression were not correlated with changes in expression of the corresponding protein. All Waghray *et al.* state is that "for most of the proteins identified, there was no appreciable concordant change at the mRNA level" (page 1337, col. 2). This statement is not relevant to Applicants' assertion of utility, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. Waghray *et al.* do not contradict Applicants' assertion that changes in mRNA expression, in general, correspond to changes in expression of the corresponding protein.

Lastly, the Examiner cites Sagynaliev *et al.*, as allegedly teaching that "it is also difficult to reproduce transcriptomics results with proteomics tools." In particular, the Examiner notes that according to Sagynaliev *et al.*, of 982 genes found to be differentially expressed in human CRC, only 177 (18%) have been confirmed using proteomics technologies. (Page 7 of the instant Office Action).

The Sagynaliev *et al.* reference, titled "Web-based data warehouse on gene expression in human colorectal cancer" (emphasis added), drew conclusions based upon a literature survey of gene expression data published in human CRC, and not from experimental data. While a literature survey

can be a useful tool to assist researchers, the results may greatly over-represent or under-represent certain genes, and thus the conclusions may not be generally applicable. In particular, Applicants note that, as evidenced by Nagaraja *et al.* and Waghray *et al.*, discussed above, the number of mRNAs examined in transcriptomics studies is typically much larger than the number of proteins examined in corresponding proteomics studies, due to the difficulties in detecting and resolving more than a small minority of all expressed proteins on 2D gels. Thus the fact that only 18% of all genes found to be differentially expressed in human CRC have been confirmed using proteomics technologies does not mean that the corresponding proteins are not also differentially expressed, but is most likely due to the fact that the corresponding proteins were not identified on 2D gels, and thus their expression levels remain unknown.

The authors of Sagynaliev *et al.* acknowledge the many technical problems in finding proteomic data for CRC that can be matched to transcriptomic data to see if the two correlate. The authors state that "results have been obtained using heterogeneous samples in particular cell lines, whole tissue biopsies, and epithelial cells purified from surgical speciements." However, "Results obtained in cell lines do not allow accurate comparison between normal and cancer cells, and the presence/absence of proteins of interest has to be confirmed in biopsies." (Page 3072, left column.) In particular, the authors specifically note that "only a single study [1] provided differential display protein expression data obtained in the human patient, using whole tissue biopsy." (Page 3068, left column, second paragraph; *see also*, Table 2.) The examiner also notes and the authors state, "For CRC, there is no publication comparing mRNA and protein expression for a cohort of genes." (Page 3077, left column, last paragraph, emphasis added.)

Applicants further note that Table 2 shows that 6 out of 8 published proteomics studies were done using 2-D PAGE. However, the authors state that "2-D PAGE or 2-D DIGE have well-known technological limitations … even under well-defined experimental conditions, 2-D PAGE parallel analysis of paired CRC samples is hampered by a significant variability." (Page 3077, left column, third paragraph.) Therefore, Applicants respectfully submit that it is well known in the art that there are problems associated with selecting only those proteins detectable by 2D gels.

Finally, the Examiner asserts that "the specification of the instant application does not teach a change in mRNA level of PRO213-1" because "[t]here are no teachings in the specification as to the differential expression of PRO213-1 mRNA in the progression of colon cancer or in response to different treatments of hormones (for example)." (Pages 7-8 of the instant Office Action).

Applicants respectfully note that the instant specification measured gene amplification, not mRNA expression. Applicants further submit that it is well known that cancers arise from the transformation of normal tissue cells to cancerous cells, thus the observed differences in gene amplification between normal and cancerous tissues are in fact the result of previously occurring changes.

Lilley *et al.*, Wildsmith *et al.* and King *et al.*

The Examiner next asserts that "the state of the art, as evidenced through textbooks and review papers, clearly establishes that polypeptide levels cannot be accurately predicted from mRNA levels." (Page 8 of the instant Office Action). In support of this assertion, the Examiner cites textbook excerpts by Lilley *et al.* and Wildsmith *et al.*, and an article by King *et al.* In particular, the Examiner cites Lilley *et al.* to the effect that "the extrapolation that changes in transcript level will also result in corresponding changes in protein amount or activity *cannot always be made*." (Page 8 of the instant Office Action, emphasis added). The Examiner cites Wildsmith *et al.* to the effect that "the gene expression data obtained from a microarray *may differ* from protein expression data." (Page 8 of the instant Office Action, emphasis added). Finally, the Examiner cites King *et al.* to the effect that "it has been established that mRNA levels do not *necessarily* correlate with protein levels." (Page 8 of the instant Office Action, emphasis added).

Applicants reiterate that the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. **The standard is not absolute certainty.** The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. The law does not require a "necessary" correlation between mRNA and protein levels. Nor is it required that protein levels can be "accurately predicted" from mRNA levels. Nowhere in these papers do the authors suggest that it is more likely than not that altered mRNA levels do not correlate with altered protein levels. On the contrary, statements such as "the extrapolation that changes in transcript level will also result in corresponding changes in protein amount or activity cannot *always* be made" imply that the mRNA/protein correlation exits in most cases.

Applicants further note that the cited papers disclose a number of successful examples of microarray applications in human disease study, which further validate Applicants' assertions. For example, Wildsmith *et al.* points out that

> one area of rapid progress using microarray technology is the increased understanding of cancer. Molecular pathologies are subgrouping cancers of tissues such as blood, skin, and breast, based on differential gene expression patterns. For example, within a small group of breast cancer tissue samples, Perou *et al.* distinguished two broad subgroups representing those expressing or alternatively lacking expression of the oestrogen receptor- α- gene. The work was not conclusive, but never has progress in this field been so rapid when compared with the previous methods of gene amplification. Another example of the impact of this technology is in the identification of two biomarkers for prostate cancer, namely hepsin and PIM1 (Dhanasekaran *et al.*, 2001). Microarray technology has also accelerated the understanding of the molecular events surrounding pulmonary fibrosis. Specially, two distinct clusters of genes associated with inflammation and fibrosis have been identified in a disease where, for years, the pathogenesis and treatment have remained unknown (Katsuma *et al.*, 2001). (Page 284).

King *et al.* disclose that microarray technology offers tremendous advantagies in human disease study. For example, the authors state that "microarrays can be expected to prove extremely valuable as tools for the study of the generic basis of complex diseases. The ability to measure expression profiles across entire genomes provides a level of information not previously attainable…Microarrays make it possible to investigate differential gene expression in normal vs. diseased tissue, in treated vs. non-treated tissue, and in different stages during the natural course of the disease, all on a genomic scale. Gene expression profiles may help to unlock the molecular basis of phenotype, response to treatment, and heterogeneity of disease." (Page 2287, column 3).

Bork *et al.*

The Examiner also refers to a paper by Bork *et al.* (Page 8 of the instant Office Action). Bork *et al.* comments generally about high-throughput technologies (which include microarrays) and in fact, validates the positive potential of such technologies by admitting that such technologies "often reveal important general trends that are impossible to realize with classical, low-throughput experimental methods, yet provide fewer insights into specific, molecular detail (see page 1, column 1, line 3-8 of the Bork article). This article comments on the limitations in the "total knowledge base" of protein function. Bork further quotes Anderson *et al.*'s

coefficient of 0.48 as the correlation between mRNA and protein expression. Anderson *et al.* was discussed in the previous Responses, where Applicants indicated that a 0.48 correlation value (about 50%) supports the contention that it is "more likely than not" that protein expression correlates well with mRNA expression. Therefore, like Anderson, Bork supports the Applicants' position that changes in mRNA levels are generally correlated with changes in protein levels.

Haynes *et al.*

The Examiner cites Haynes *et al.* to the effect that "[p]rotein expression levels are not predictable from the mRNA expression levels." (Page 8 of the instant Office Action). Applicants respectfully point out that Haynes *et al.* never indicate that the correlation between mRNA and protein levels does not exist. Haynes *et al.* only state that "protein levels cannot be *accurately* predicted from the level of the corresponding mRNA transcript" (See page 1863, under Section 2.1, last line, emphasis added). This result is expected, since there are many factors that determine translation efficiency for a given transcript, or the half-life of the encoded protein. Not surprisingly, Haynes *et al.* concluded that protein levels cannot always be accurately predicted from the level of the corresponding mRNA transcript in a single cellular stage or type when looking at the level of transcripts across different genes.

Importantly, Haynes *et al.* did not say that for a single gene, a change in the level of mRNA transcript is not positively correlated with a change in the level of protein expression. Applicants have asserted that increasing the level of mRNA for a particular gene leads to a corresponding increase for the encoded protein. Haynes *et al.* did not study this issue and says absolutely nothing about it. One cannot look at the level of mRNA across several different genes to investigate whether a change in the level of mRNA for a particular gene leads to a change in the level of protein for that gene. Therefore, Haynes *et al.* is not inconsistent with or contradictory to the utility of the instant claims, and offers no support for the PTO's rejection of Applicants' asserted utility.

Furthermore, Applicants note that contrary to the Examiner's statement, Haynes teaches that "*there was a general trend* but no strong correlation between protein [expression] and transcript levels" (See page 1863, under Section 2.1, emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein amongst *most* of the 80 yeast proteins studied but the correlation is not linear, hence the authors suggest that one cannot *accurately*

predict protein levels from mRNA levels. In fact, very few data points deviated or scattered away from the expected normal or showed a lack of correlation between mRNA: protein levels. Thus, the Haynes data meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Haynes *et al.*

Haynes *et al.* may teach that protein levels cannot be "accurately predicted" from mRNA levels in the sense that the exact numerical amounts of protein present in a tissue cannot be determined based upon mRNA levels. Applicants respectfully submit that the PTO's emphasis on the need to "accurately predict" protein levels based on mRNA levels misses the point. The asserted utility for the claimed polypeptides is in the diagnosis of cancer. What is relevant to use as a cancer diagnostic is relative levels of gene or protein expression, not absolute values, that is, that the gene or protein is differentially expressed in tumors as compared to normal tissues. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predict protein overexpression. A showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required.

Madoz Gurpide *et al.*

The Examiner cites Madoz Gurpide *et al.* to the effect that "[f]or most of the published studies, it is unclear how well RNA levels reported correlate with protein levels." (Page 9 of the instant Office Action). Applicants respectfully point out that Madoz Gurpide *et al.* state only that is it "unclear" how well RNA levels reported correlate with protein levels, not that the levels do not correlate. In support of this assertion, the authors cite only a single reference, the same paper by Chen *et al.* previously cited by the Examiner, which Applicants have already discussed. Madoz Gurpide *et al.* also acknowledge that DNA microarray studies "**justify the use of this technology for uncovering patterns of gene expression that are clinically informative**" (page 53; emphasis added).

Applicants respectfully submit that while proteomics is indeed a complementary technology to DNA microarrays, this does not mean that proteomic experiments are required in addition to measurements of mRNA levels to determine protein expression. The cited papers make clear that proteomic techniques are useful to obtain information beyond expression levels, such as the protein's activation state, posttranslational modifications, and subcellular localization. For example, Madoz-Gurpide *et al.* explain that mRNA expression alone does not

provide information regarding "activation state, post-translational modification or localization of corresponding proteins" (page 168, col. 1). Haynes et al., as quoted in the instant Office Action, states that "only the direct analysis of mature protein products can reveal their correct identies, their relevant state of modification and/or association, and their amounts." (Pages 8-9 of the instant Office Action).

While this additional information may be useful in elucidating the detailed biological function of a protein, it is not required to establish utility of a protein as a marker for cancer. The PRO213-1 polypeptide can be used in cancer diagnosis without any knowledge regarding the function or cellular role of the polypeptide. Applicants submit that the law clearly states that "it is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works." *Newman v. Quigg*, 11 U.S.P.Q.2d 1340 (Fed. Cir. 1989). Accordingly, the disclosure or identification of the mechanism by which PRO213-1 is associated with cancer is not required in order to establish the patentable utility of the claimed PRO213-1 polypeptides. Thus while Madoz-Gurpide *et al.* note that it is "more difficult to develop an understanding of disease at a mechanistic level using DNA microarrays," (page 53) this is not relevant to Applicants' assertions of utility, since, as discussed above, it is not necessary to understand how or why an invention works in order to demonstrate utility.

The Patent Office has failed to meet its initial burden of proof that Applicant's claims of utility are not substantial or credible. The arguments presented by the Examiner in combination with the previously cited Pennica, Gygi, Lian, Fessler, Chen and Anderson papers, as well as the newly cited Nagaraja, Waghray, Sagynaliev, Lilley, Wildsmith, King, Bork, Haynes, and Madoz-Gurpide papers, do not provide sufficient reasons to doubt the statements by Applicants that PRO213-1 has utility. As previously discussed, the law does not require the existence of a "necessary" correlation between mRNA and protein levels. Nor does the law require that protein levels be "accurately predicted." According to the authors themselves, the data in the above cited references confirm that there is a general trend between protein expression and transcript levels, which meets the "more likely than not standard" and show that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's reasoning is based on a misrepresentation of the scientific data presented in the above cited reference and application of an improper, heightened legal standard. In fact, contrary to what the Examiner

contends, the art indicates that, if a gene is overexpressed in cancer, it is more likely than not that the encoded protein will also be expressed at an elevated level.

It is "more likely than not" for amplified genes to have increased mRNA and protein levels

Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Applicants' Response filed October 4, 2004) collectively teach that in general, gene amplification increases mRNA expression. Second, the Declaration of Dr. Paul Polakis, submitted with Applicants' Response filed October 4, 2004, shows that, in general, there is a correlation between mRNA levels and polypeptide levels.

Furthermore, in their Preliminary Amendment filed July 7, 2006, Applicants submitted a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B. Exhibit B of the Polakis II Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA."

The Examiner asserts that the second Polakis Declaration is insufficient to overcome the utility rejection because PRO213-1 does not appear in the table (Exhibit B), and allegedly "it is not clear how the clones appearing in the table compare to PRO213-1, or if the results presented in the table report on the results of the same assay as presented in Example 30 of the instant specification." (Pages 15-16 of the instant Office Action).

Applicants respectfully note that the Polakis Declaration describes the results of microarray experimentation, while Example 114 (not Example 30) of the specification discloses gene amplification data. Thus the Examiner's attempt to contrast the methodology of the two types of experiments is misplaced.

Applicants further respectfully submit that, as discussed in the Preliminary Amendment filed July 7, 2006, the standard for utility is more likely than not. Dr. Polakis' Declarations provide evidence, in the form of statements by an expert in the art, that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell." The PRO213-1 gene was found to be amplified in lung and colon tumors. As discussed above and in Applicants' previous Responses, one of ordinary skill in the art would therefore expect the PRO213-1 mRNA to be overexpressed in the same human lung and colon tumor samples. Accordingly, one of ordinary skill in the art would understand that the PRO213-1 polypeptide would be expected (more likely than not) to be overexpressed in human lung and colon tumor samples relative to their normal human tissue counterparts, as are the majority of other molecules tested.

Applicants have also previously submitted, with their Supplemental Preliminary Amendment filed September 6, 2006, a Declaration by Dr. Randy Scott ("the Scott Declaration"). Dr. Scott was a co-founder of Incyte Pharmaceuticals, Inc., the world's first genomic information business, and is currently the Chairman and Chief Executive Officer of Genomic Health, Inc., a life sciences company located in Redwood City, California, which provides individualized information on the likelihood of disease recurrence and response to certain types of therapy using gene expression profiling. Based on his more than 15 years of personal experience with the DNA microarray technique and its various uses in the diagnostic and therapeutic fields, and his familiarity with the relevant art, Dr. Scott unequivocally confirms that, as a general rule, there is a good correlation between mRNA and protein levels in a particular tissue.

As stated in paragraph 8 of the Scott Declaration:

> DNA microarray analysis has been extensively used in drug development and in diagnosis of various diseases. ..... Due to its importance in drug discovery and in the field of diagnostics, microarray technology has not only become a laboratory mainstay but also created a world-wide market of over $600 million in the year of 2005. A long line of companies, including Incyte, Affymetrix, Agilent, Applied Biosystems, and Amersham Biosciences, made microarray technology a core of their business.

In paragraph 10 of his Declaration, Dr. Scott explains the reasons for the wide-spread use and impressive commercial success of this technique, stating:

> One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, **it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue**. Therefore, diagnostic markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein expression levels. (emphasis added).

The Declaration, which is based on Dr. Scott's unparalleled experience with both the microarray technique and its industrial and clinical applications, supports Applicants' position that microarray technology is not only mature, reliable and well-accepted in the art, but also has been extensively used in drug development and in diagnosis of various diseases and produced enormous commercial success. Therefore, if a gene, such as the gene encoding the PRO213-1 polypeptide, has been identified to be over-expressed in a certain disease, such as lung or colon cancer, it is more likely than not that the protein product is also overexpressed in the disease.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[1] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument."[2] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an Examiner."[3] Applicants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[4] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." The

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[1] *In re Rinehart,* 531 F.2d 1084, 189 U.S.P.Q. 143 (C.C.P.A. 1976); *In re Piasecki,* 745 F2d. 1015, 226 U.S.P.Q. 881 (Fed. Cir. 1985).

[2] *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir 1996) (quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992)).

[3] *Id.* at 1583.

[4] Part IIB, 66 Fed. Reg. 1098 (2001).

statement in question from an expert in the field (the Scott Declaration) states: "elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue." Therefore, barring evidence to the contrary regarding the above statement in the Scott Declaration, this rejection is improper under both the case law and the Utility guidelines. As discussed in detail above, the various articles cited in the Office Action do not provide such countervailing evidence.

Furthermore, Applicants have argued in their Preliminary Amendment filed July 7 2006, that numerous references demonstrate that mRNA levels correlate to protein expression levels, and have submitted over a hundred such references in their Information Disclosure Statement filed July 7, 2006. These references have not been addressed in the instant Office Action.

Based on the above arguments, Applicants have clearly demonstrated a credible, specific and substantial asserted utility for the claimed PRO213-1 polypeptides, for example, as diagnostic markers for lung and colon tumors. Further, based on this utility and the disclosure in the specification, one skilled in the art at the time the application was filed would know how to use the claimed polypeptides.

Applicants therefore respectfully request withdrawal of the rejections of Claims 58-63, 69 and 70 under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

II. Claim Rejections Under 35 U.S.C. §112, First Paragraph (Written Description)

Claims 58-62, 69 and 70 remain rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking adequate written description for the claimed variant polypeptides having at least 80-99% identity to amino acid residues 35-273 of SEQ ID NO:506, wherein the nucleic acid encoding the polypeptide is amplified in colon or lung tumors.

Applicants respectfully maintain the position that that Claims 58-62 and 69-70 satisfy the written description requirement under 35 U.S.C. §112, first paragraph, for the reasons previously set forth in Applicants' Responses filed on October 4, 2004, May 23, 2005, and November 18, 2005, and in the Preliminary Amendment filed July 7, 2006.

The Examiner asserts that Applicants' arguments are not considered persuasive because "80% identity to a described sequence is not a true structure. It is not disclosed which of the 80% amino acids are important for activity." (Page 14 of the instant Office Action). Applicants respectfully submit that this statement is not consistent with Example 14 of the Synopsis of

Application of Written Description Guidelines issued by the U.S. Patent Office, which makes clear that a defined degree of homology to a reference sequence, when combined with known procedures for making variant proteins and an assay for detecting the functional activity of the variants, is sufficient to provide adequate written description for the variant polypeptides. There is simply no requirement in the Guidelines for a disclosure of precisely which amino acids are important for activity.

The Examiner further asserts that "being overexpressed in tumors is not a functional property, but a characteristic." (Page 14 of the instant Office Action). Applicants submit that the law is clear that "[a] functional limitation is an attempt to define something by what it does, rather than by what it is (e.g., as evidenced by its specific structure or specific ingredients)."[5] "A functional limitation is often used in association with an element, ingredient, or step of a process to define a particular capability or purpose that is served by the recited element, ingredient or step."[6] Accordingly, overexpression of the claimed polypeptides in lung or colon tumor cells is a functional limitation which indicates the functional purpose (i.e., use in the diagnosis of cancer) of the claimed polypeptides.

Applicants therefore respectfully request that the Examiner reconsider and withdraw the written description rejection of Claims 58-62 and 69-70 under 35 U.S.C. §112, first paragraph.

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[5] *In re Swinehart*, 439 F.2d 210, 169 U.S.P.Q. 226 (C.C.P.A. 1971).

[6] M.P.E.P. 2173.05(g).

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned agent at the telephone number shown below.

Although no fees are due, the Commissioner is hereby authorized to charge any fees, including any fees for extension of time, or credit overpayment to Deposit Account No. **08-1641**, referencing Attorney's Docket No. **39780-2630 P1C4**. Please direct any calls in connection with this application to the undersigned at the number provided below.

Respectfully submitted,

Date: January 26, 2007

By: _____
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